REVIEW

Development and Fundamental Characteristics of a Human Gastric Digestion Simulator for Analysis of Food Disintegration

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Abstract

Gastric digestion is the major digestion process in humans and is strongly affected by both physical and chemical digestion. *In vitro* approaches using different gastric digestion models have received a great deal of attention in several scientific and industrial fields, including food science and technology, due to experiments being conducted under various conditions and with better reproducibility of the experiment data. The development of simple *in vitro* gastric digestion devices that enable quantitative consideration of the influence of gastric peristalsis has been necessary for simulating and analyzing the disintegration of solid foods in the stomach. The authors and co-workers recently developed a human gastric digestion simulator (GDS) that simplifies the antrum geometry, is capable of simulated gastric peristalsis, and which enables direct observation of the disintegration of food particles in the gastric contents. This article provides a brief overview of our findings regarding the GDS. First, the concept and development of the GDS is introduced. The disintegration characteristics of representative (model) foods using the GDS are described next, providing insights into the digestion processes influenced by gastric peristalsis. After further improvement, the GDS is expected to offer potential as a tool for designing novel nutraceutical and functional foods for which digestibility is well controlled.

Discipline: Food
Additional key words: Antrum, direct observation, human stomach, *in vitro* device, peristalsis

Introduction

The stomach is a major digestive organ in the human body and plays important roles in disintegrating ingested foods into smaller sizes, and making the nutrients and functional components embedded in them accessible to digestion and adsorption. The human digestion process can be divided into physical, chemical, and biological processes. The oral digestion process that includes mastication and swallowing is greatly influenced by the physical digestion process. The small-intestinal digestion process depends on the chemical digestion process as well as the physical digestion process. The biological digestion process entailing a sort of microbial fermentation occurs in the large intestine. Unlike the processes described above, the gastric digestion process is strongly affected by both the physical and chemical digestion processes.

The stomach has functions such as for storing a bolus (a masticated mixture of food particles and saliva) in its upper part (body), mixing, breaking down, and grinding of the gastric contents, induced by peristalsis that is generated on the gastric wall, chemical and enzymatic reactions of the gastric contents with gastric fluid secreted from the gastric wall, and the emptying of gastric digesta (chyme) from the gastric outlet (pylorus) (Kong et al. 2008). Food particles in the bolus usually undergo dramatic changes as a result of the physical and chemical digestion processes. It is therefore important to simulate and analyze the gastric digestion process for a better understanding of key gastric digestion phenomena and the design of novel foods with adequately controlled digestibility.

Gastric digestion studies of foods have been conducted

using *in vivo*, *in vitro*, and *in silico* approaches (Kong et al. 2008, Ferrua & Singh 2010, McClements & Li 2010, Guerra et al. 2012). The *in vivo* approach provides the most reliable information about gastric digestion phenomena. In particular, the use of magnetic resonance imaging (MRI) provided quantitative data on human gastric peristalsis as well as information about the breakdown of food particles in the human stomach (Marciani et al. 2001a, 2001b, Pal et al. 2004). However, it is difficult to investigate various conditions due to restrictions on the number of subjects in the *in vivo* approach. The *in silico* approach using computers is useful for simulating and analyzing the flow phenomena of liquid gastric contents induced by peristaltic motion on the gastric wall (Pal et al. 2004, Kozu et al. 2010, Ferrara et al. 2010, Imai et al. 2013). The *in vivo* and *in silico* approaches are capable of analyzing the physical gastric digestion process to some extent, but make it difficult to analyze the disintegration of gastric contents containing solid food particles in detail.

The *in vitro* approach has frequently employed simple shaking methods using test tubes or flasks (McClements & Li 2010). Continuous-stir tank reactors capable of secreting a simulated digestive fluid and emptying of the digested sample have been proposed by several research groups (Mainville et al. 2005, Van Aken et al. 2011). However, these methods cannot reasonably consider the physical forces caused by human gastric peristalsis. Complex *in vitro* gastric (and gastrointestinal) digestion devices equipped with segmentation or peristalsis have been developed by research groups in Europe (Minekus et al. 1995, Mercuri et al. 2008). These devices realize the automated operation of *in vitro* gastric digestion experiments. In recent years, there have been growing demands for the development of simpler *in vitro* gastric devices for food applications. Kong et al. (2010) developed a human gastric simulator (HGS) that models the entire stomach. The HGS is equipped with quantitatively simulated peristaltic motion generated in the lower part of the stomach called antrum contraction waves (ACWs). *In vitro* gastric digestion devices are reviewed in detail elsewhere (Guerra et al. 2012).

The disintegration of foods in the presence of ACWs has not been well understood. A possible approach to achieving a detailed understanding of these behaviors is to develop a novel *in vitro* gastric digestion device capable of visualizing the motion of gastric contents. The authors recently developed a human gastric digestion simulator (GDS) that enables direct real-time observation of the disintegration of gastric contents in the presence of quantitatively simulated ACWs (Kozu et al. 2014a). This review will introduce the concept and development of the GDS, and the *in vitro* gastric digestion characteristics of representative (model) foods using the GDS.

Development of the GDS

The GDS developed by the authors' research group utilizes concepts including the simplification of gastric structure, quantitative simulation of gastric peristalsis, and visualization of the motion of gastric contents. Figure 1 illustrates the concept of developing the GDS as a new *in vitro* gastric digestion model (Kozu et al. 2014a, b).

Prior to the GDS, a human gastric fluid simulator (GFS) was developed to investigate intragastric flow induced by antrum contraction waves (ACWs) (Kozu et al. 2014b). The key components of the GFS, which simplifies the antrum structure and function, include a gastric vessel consisting of transparent plastic walls and deformable rubber walls, and plastic rollers that generate ACWs (Fig. 1B). The height and speed of the ACWs are controlled based on *in vivo* data from healthy human adults obtained using magnetic resonance imaging (MRI) (Pal et al. 2004). The use of particle image velocimetry (PIV) enabled the visualization of flow patterns of intragastric contents with or without spherical particles, as model food particles, in the GFS. The ACWs that act on the gastric walls induce two characteristic flows of liquid intragastric contents: retropulsive flow in and behind the occluded region, and eddy flow near the wall behind the occluded region. These flow patterns were confirmed by computational fluid dynamics (CFD) simulation using the GFS model. Pal et al. (2004) also reported the existence of both patterns using a lattice Bolzmann simulation with a whole-stomach model. The flow-field profiles in the GFS demonstrated shear flow around the particles in intragastric contents. The shear rate acting on the intragastric contents was highest near the rubber wall in the occluded region and/or on the upstream side of the particles. However, maximum shear rates were on the order of 10 s^{-1}, strongly indicating a low shear force acting on the intragastric contents induced by ACWs. The findings obtained using the GFS were utilized for developing the GDS for *in vitro* gastric digestion experiments.

The authors developed a new *in vitro* gastric digestion model called the GDS that can conduct and directly observe *in vitro* gastric digestion experiments in the presence of simulated gastric peristalsis (Fig. 2) (Kozu et al. 2014a). Figure 1C depicts the key components of the GDS, consisting of a gastric vessel that models the antrum and rollers that quantitatively generate ACWs at a specific interval. The gastric vessel, with a total volume of 550 mL, has dimensions similar to those of the human antrum and is equipped with transparent parallel windows for monitoring the disintegration of food particles during digestion in real time. In addition to speed, the interval (e.g., a few cycles per minute) of ACWs is also controllable by adjusting the position of the rollers. The temperature inside and around the gastric vessel is kept at 37°C during the experiments

using a heating unit. In principle, the gastric vessel has an inlet (top side) for supplying simulated gastric fluid, and an outlet that models the pylorus (bottom side) for emptying of the digested samples. Our previous GDS work was conducted without the operations described above in order to more simply evaluate the influence of gastric peristalsis on the disintegration of (model) food particles soaked in a gastric fluid (Kozu et al. 2014a, 2015). GDS results considering the secretion of gastric fluid and emptying of gastric digesta (chyme) have recently reported (Kozu et al. 2016).

The ACWs generated by the rollers in the GDS can apply physical forces to the food particles. The physical forces induced by the ACWs must be adjusted to reasonably simulate the disintegration of food particles during the GDS experiments. The GDS equipment currently uses sponge rollers with a plastic core to adequately reduce the physical
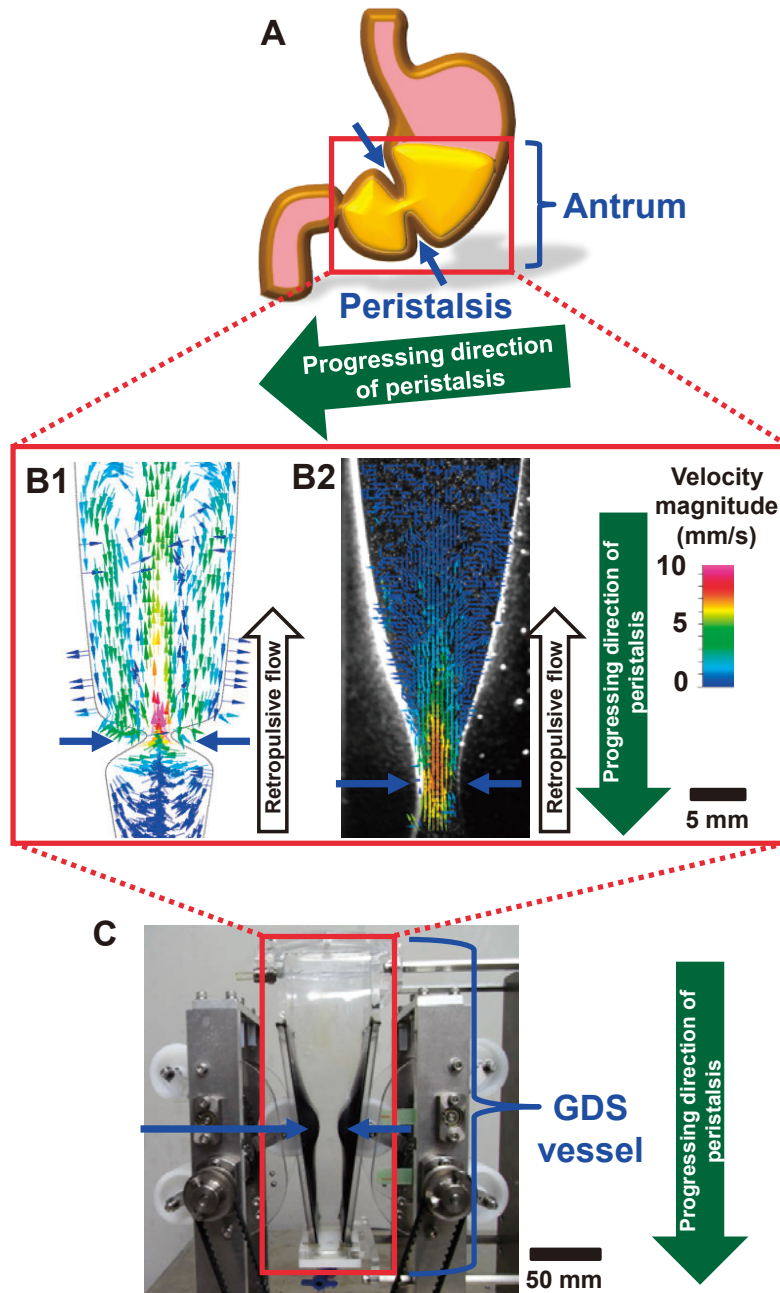

Fig. 1. Development of the Gastric Digestion Simulator (GDS).
    A: The part inside the rectangle is the antrum, our principal point of focus where gastric peristalsis mainly occurs. B: Intragastric flow studies using B1—a computational antrum model (Kozu et al., 2010) and B2—an experiment model called Gastric Flow Simulation (Kozu et al. 2015). C: Developed Gastric Digestion Simulator in reference to the two intragastric flow models mentioned above (Kozu et al. 2014a). All three models are able to simulate gastric peristalsis. Horizontal blue arrows in B and C denote simulated peristalsis.

forces acting on food particles. A few *in vivo* studies have estimated the compression force acting on agar gel particles or tablets (Marciani et al. 2000, Kamba et al. 2001). The estimated compression force ranged from 0.65 to 1.9 N.

As stated above, the GDS is advantageous due to the combination of quantitatively simulated gastric peristalsis and direct observation of the disintegration of food particles when considering physical and chemical gastric digestion. The next section introduces the *in vitro* digestion characteristics of three different types of (model) foods using the GDS.

In vitro digestion characteristics of food particles using the GDS

1. *In vitro* gastric digestion procedures

GDS experiments are normally conducted using (model) food particles with a size of 5 mm or less (Kozu et al. 2014a, 2015, Wang et al. 2015). For instance, agar gel and *Tofu* larger than this size range were cut into 5-mm cubes before each experiment. This particle size was selected mainly due to the larger portion of particles after mastication (Jalabert-Malbos et al. 2007). Previous research using a different gastric model also selected similar particle sizes (Kong & Singh 2008, 2009). Moreover, the particle sizes selected for GDS experiments are useful for easier direct observation of particle disintegration in a gastric vessel (Fig. 3).

The experimental procedures using the GDS are briefly described below. To start the GDS experiment, a simulated bolus consisting of (model) food particles and simulated saliva (pH 7) is supplied in a gastric vessel containing a simulated gastric fluid (Kozu et al. 2014a, 2015). The simulated saliva contains 0.117 g/L NaCl, 0.14 g/L KCl, 2.1 g/L NaHCO$_3$, and 2.0 g/L α-amylase in Milli-Q water. The simulated gastric fluid, adjusted at pH 1.3 using diluted HCl, contains 8.775 g/L NaCl and 1.0 g/L pepsin in Milli-Q water. Each GDS experiment is conducted at 37°C up to 180 min. in the presence of simulated ACWs at a progressing speed of 2.5 mm/s and a generation frequency of 1.5 cycle/min (Pal et al. 2004). The disintegration behavior of (model) food particles can be monitored and recorded
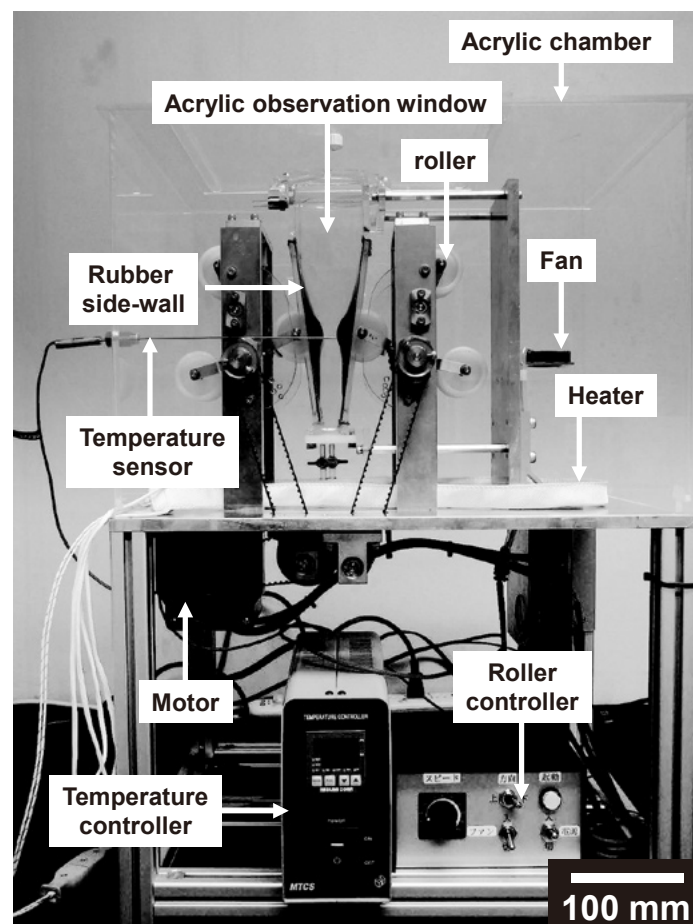

Fig. 2. Photograph of the GDS including the gastric vessel, roller control system, and temperature control system (Kozu et al. 2014a).

using a video camera.

Flask-shaking experiments as commonly employed by *in vitro* gastric digestion methods are conducted based on the procedure described by Wang et al. (2013) with slight modifications. Briefly, a mixture of (model) food particles, simulated saliva, and simulated gastric fluid is introduced into an Erlenmeyer flask, being subsequently incubated at 37°C up to 180 min at a shaking frequency of 115 strokes/min. It should be noted that a harmonized static *in vitro* digestion method for food was recently proposed by Minekus et al. (2014).

2. Measurements and analysis

The particle size distribution after *in vitro* digestion experiments is determined by measuring the weight of each size fraction after classification using the sieves of
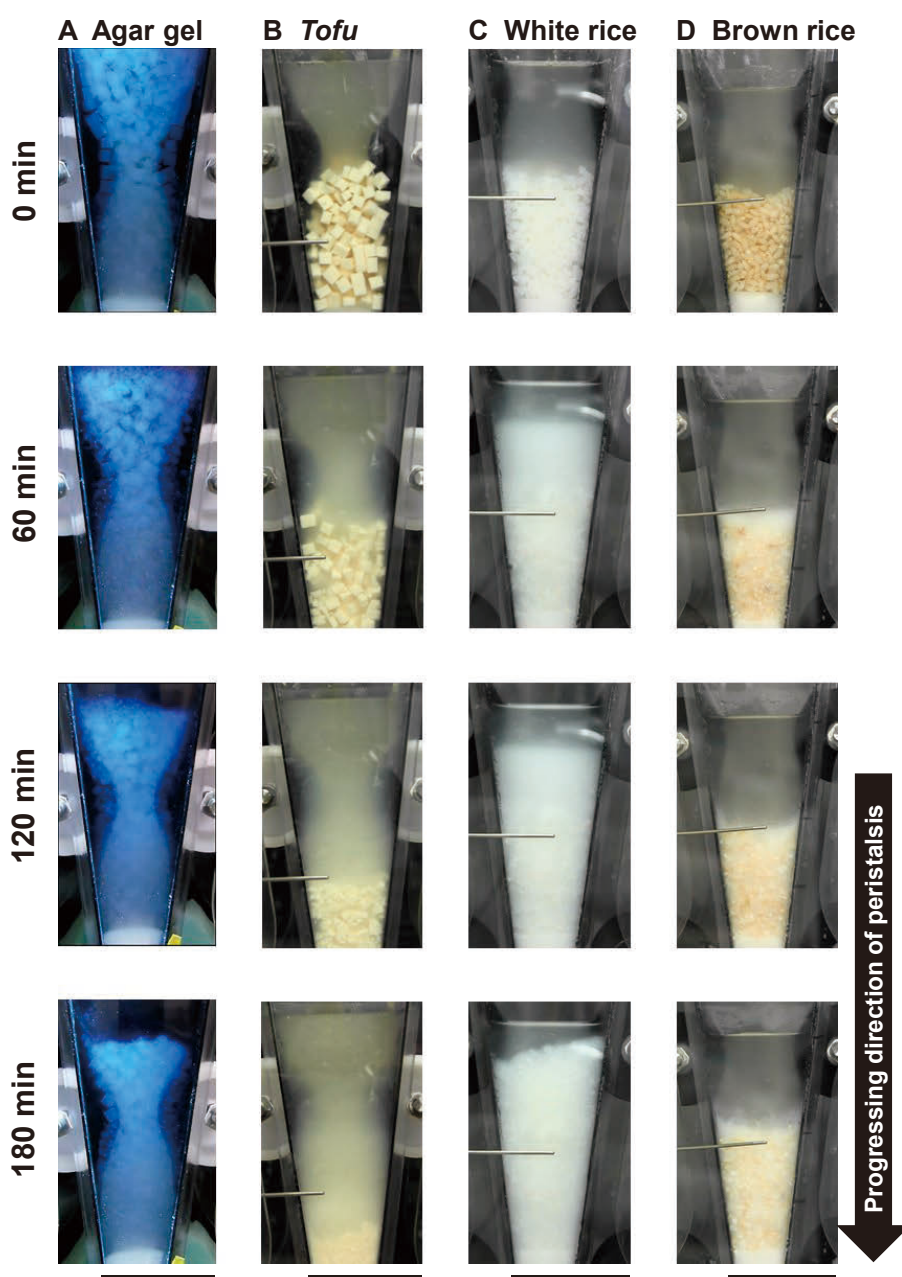

Fig. 3. Direct observation of food digestion using the GDS.
A: Agar gel (Kozu et al. 2015); B: *Tofu* (Kozu et al. 2014a); C: White rice (Wang et al. 2015); D: Brown rice (Wang et al. 2015). Each black line below the photographs represents a 50 mm scale bar. A temperature sensor is presented as a horizontal metallic bar in each image of B to D.

four different mesh sizes: 0.60, 1.18, 2.36, and 3.35 mm. The dry weight of each size fraction is generally measured for digested particles (Kong & Singh 2009, Kozu et al. 2014a). Prior to this measurement, the particles of each size fraction are dried to a constant weight in a vacuum oven at an elevated temperature. In contrast, the wet weight of each size fraction is measured for the digested particles of high water content hydrogels (Kozu et al. 2015). The shape of the classified particles is analyzed by using their photographs.

3. Agar gel particles

Agar gels were selected as model foods for a basic investigation of the *in vitro* digestion characteristics of food particles using the GDS (Kozu et al. 2015). The major reasons for using agar gels include their indigestibility by gastric enzymes (mainly pepsin), easy control of their particle size and hardness, and a simple gel composition. In other words, the use of agar gel particles is beneficial for focusing on physical gastric digestion.

Prior to each GDS experiment, agar gel particles (5-mm cubes) are mixed with simulated saliva (pH 7) at 37°C to consider the influence of mastication. The GDS experiment begins when this mixture is added to a simulated gastric fluid containing pepsin (pH 1.2) in the gastric vessel. Figure 3A depicts the variations of 1.5wt% agar gel particles during the GDS experiment. The GDS experiments were conducted for a maximum of 180 min, as the bolus ingested in the human stomach is mostly digested within this time (Camilleri et al. 1985). The agar gel particles gradually and randomly disintegrate in the presence of periodically generated ACWs. This particle size reduction led to denser packing and lower packing height. We observed that the breakdown of agar gel particles is driven by the compression force and shear stress between the particles, induced by ACWs. As expected from the GFS data, the fluid shear force seemed not to affect the breakdown of particles. This was confirmed by *in vitro* gastric digestion using a flask-shaking method, which mainly applies fluid shear stress to food particles. The direct observation results (Fig. 3A) demonstrate that our GDS first realizes a real-time analysis of the disintegration of food particles during *in vitro* gastric digestion in the presence of ACWs. This visual information is useful for a better understanding of gastric digestion phenomena and for developing a new class of foods whose digestibility can be controlled on demand.

The GDS can also provide particle-size distribution data after *in vitro* gastric digestion experiments. Figure 4 shows the size distributions of agar gel particles before and after the GDS experiments. The digested particles are fractionated into four different size ranges. The gastric outlet called the pylorus has an inner diameter of about 2 mm (Kong et al. 2008), and digested particles smaller than

the pylorus are usually emptied into the duodenum. The weight ratio of the particles exceeding 2.36 mm decreased with *in vitro* gastric digestion using the GDS. Over 80% (in wet weight) of the particles was smaller than the pylorus size after 180 min of the GDS experiment (i.e., ACWs may possibly disintegrate particles into sizes that can be emptied from the pylorus). The flask-shaking data (Fig. 4) clearly demonstrate that the size distribution of agar gel particles hardly varies in the absence of ACWs. Though conventional shaking methods using a flask or test tube are suitable for liquid foods such as emulsions and beverages, an *in vitro* gastric digestion method that quantitatively considers ACWs must be used for solid and semi-solid foods. The GDS is a promising device for simulating and analyzing gastric digestion phenomena of solid and semi-solid foods.

The physical properties of foods are major factors affecting digestion processes, including gastric digestion in humans. Our GDS study was also conducted using agar gel particles with different fracture stresses (56 to 219 kPa) and similar fracture strains (about 30%). Interestingly, the size distributions of the digested particles were not significantly affected by the compression force induced by ACWs, suggesting that hardness is not a major parameter affecting the gastric digestion of food particles, except perhaps those of rigid foods. The fracture strain as an indicator of elasticity is assumed to remarkably affect the disintegration of food particles during gastric digestion, while the agar gels used in our previous study had similar low fracture-strain values. Further investigation is needed to understand the disintegration of foods with different fracture strains during *in vitro* digestion using the GDS.

4. *Tofu* (soybean curd)

Tofu is a food rich in soy protein that can be readily cut into cubes of the desired size. The GDS experiments using *tofu* were expected to analyze the disintegration of food particles driven by both physical and chemical gastric digestion processes (Kozu et al. 2014a). *Kinugoshi-Tofu* purchased at a local market was mainly used as the *tofu* sample for the GDS study. The *tofu* particles (5-mm cubes) used in each GDS experiment were the same size as the agar gel particles depicted in Fig. 3B (0 min).

To start the GDS experiment, a simulated bolus consisting of *tofu* particles and simulated saliva was supplied in a gastric vessel containing a simulated gastric fluid. A gradual and random breakdown of the *tofu* particles was observed from the beginning of the GDS experiment, regardless of their position in the gastric vessel (Fig. 3B). The upper portion of the gastric contents is initially composed of the simulated gastric fluid. This portion was becoming turbid within the first 60 min of digestion, unlike the results obtained using agar gel particles (Fig. 3A). A further increase in turbidity was observed until the GDS
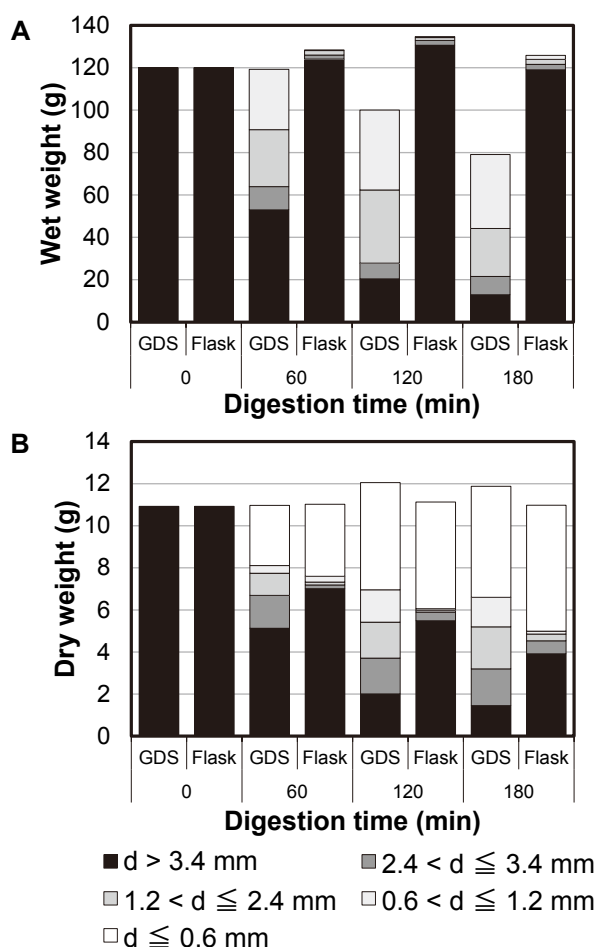
Fig. 4. Size distribution change of (A) agar gel (Kozu et al. 2015) and (B) *tofu* (Kozu et al. 2014b) particles during digestion experiments. Vertical axes represent (A) the wet weight and (B) the dry weight of the sieved fraction. The legends below the horizontal axis indicate the two kinds of digestion experiments. GDS: GDS digestion experiment. Flask: Flask-shaking digestion experiment. Note that "d" below the graphs denotes the sieve mesh-size.

experiments terminated. This increase in turbidity can be attributed to the presence of *tofu* microparticles discharged from the surface of the *tofu* particles.

This surface phenomenon is assumed to occur due to the combined effect of an enzymatic reaction in the presence of pepsin and the shear flow of the simulated gastric fluid. The results using a flask-shaking method also demonstrate that *tofu* particles become smaller more quickly as the fluid shear force increases. The *tofu* particles disintegrated using the GDS had random shapes, whereas those disintegrated by flask-shaking retained their initial cubic shape, but had smoothed edges. Moreover, the size distributions of disintegrated *tofu* particles reflected the difference in their disintegration behavior (Kozu et al. 2014a). The weight ratio

of *tofu* particles smaller than the pylorus size reached 73% for the GDS and 59% for flask-shaking at 180 min. The *in vitro* gastric digestion results using *Kinugoshi-Tofu* strongly indicate that both physical and chemical digestion processes must be reasonably considered when simulating and analyzing the disintegration of solid and semi-solid food particles. The direct observation of *tofu* particles during the GDS experiments provides useful insights into their disintegration in real time through a combination of physical and chemical digestion. The degree of protein hydrolysis by pepsin during the GDS experiments is an important factor in chemical digestion, and could be investigated by a future study.

5. Cooked white rice and brown rice

White rice and brown rice have structures more complex than agar gel and *tofu*, which have homogenous structures. Rice grains are rich in carbohydrates and contain proteins, lipids, vitamins, minerals, and dietary fiber (a higher amount in brown rice grains). About half of the world's current population lives on rice, mainly in Asian countries including Japan, as well as in some African and South American countries. A few papers have reported *in vivo* data on the disintegration of cooked rice in the stomach of small pigs (Bornhorst et al. 2012, 2013), making it possible to compare *in vivo* and *in vitro* gastric digestion results. The authors therefore used short-grain white rice and brown rice (*Koshihikari*) purchased at a local market for the GDS study (Wang et al. 2015).

The simulated bolus used for the GDS experiments was prepared by simply mixing cooked white rice or brown rice with simulated saliva. The GDS experiment using cooked rice is based on that using agar gel (Kozu et al. 2015) and *tofu* (Kozu et al. 2014a) with slight modifications. Figure 3C depicts the typical digestion of cooked white rice grains in a gastric vessel. Cooked white rice was digested in a more complex manner in the gastric vessel in the presence of ACWs. First, the cooked white rice grains quickly swelled due to a diffusion of simulated gastric fluid into the grains for the first 40 min of gastric digestion, and then the volume of the digested fraction containing digested grains increased by about 70% after 40 min. This swelling was unreported in the GDS experiments using agar gels and may indicate that *tofu* quickly triggers satiety. It should be noted that there are other foods that could swell during gastric digestion. Secondly, a gradual breakdown of the cooked white rice grains was observed during the whole gastric digestion period. This breakdown in the presence of ACWs is considered to be driven by cracking due to compression and/or friction. Thirdly, as shown in Fig. 3C, the upper portion of the gastric contents was becoming more turbid after 180 min of the GDS experiment, which is analogous to the GDS result using *tofu* (Fig. 3B). The discharge of rice starch granules into a simulated gastric fluid would cause

such an increase in turbidity, as numerous microparticles with sizes approximating those of rice starch granules were detected in particle size measurements. The liquid fraction of the gastric contents became more viscous and looked like a diluted rice starch paste. Our GDS results indicate that the disintegration of cooked white rice in the stomach is a complex process driven by physical and chemical gastric digestion.

Figure 3D depicts the typical digestion of cooked brown rice grains using the GDS. Cooked brown rice also demonstrated complex *in vitro* gastric digestion, the same as that for cooked white rice (Fig. 3C). Swelling of the cooked brown rice grains was considerably slower than that of the cooked white rice grains, as the presence of bran layers delays the discharge of simulated gastric fluid. The volume of the digested fraction increased by < 40% even after 180 min of gastric digestion. Cooked brown rice grains also broke down slowly due to the presence of their fibrous bran layers. The liquid in the gastric contents was less turbid, as the bran layers that cover rice endosperm may suppress the discharge of rice starch granules into the simulated gastric fluid. Thus, the bran layers of cooked brown rice resulted in a remarkably slower disintegration of grains during the GDS experiments.

The difference in the *in vitro* gastric digestibility of cooked white rice and brown rice was in agreement with the particle size fraction data taken after their gastric digestion using the GDS. For instance, the weight ratio of digested rice particles smaller than the pylorus size was 59% for cooked white rice and 32% for cooked brown rice. It is also noteworthy that the weight ratio of digested rice microparticles reached 51% for cooked white rice and 28% for cooked brown rice. These particle size fraction data indicate that the discharge of rice starch granules into the simulated gastric fluid cannot be considered negligible for *in vitro* gastric digestion.

In vivo data on the properties of pig gastric chyme containing cooked white rice or brown rice was reported by Bornhorst et al. (2012, 2013). The *in vivo* gastric digestibility of cooked white rice and brown rice was similar to our GDS results, in terms of the breakdown of rice grains. Moreover, a large amount of digested cooked rice remained in the pig stomach after 120 min of digestion (Bornhorst et al. 2012). Although the GDS experiments were conducted without considering the supply of simulated gastric fluid and the emptying of gastric digesta, the findings obtained using the GDS are considered reasonable.

Conclusions and outlook

A novel GDS that simplifies the antrum structure and function enables quantitative simulation of gastric peristalsis and direct real-time observation of the gastric digestion of foods. A combination of these features was first realized by our development of the GDS. The GDS has provided useful information about complex food digestion processes in the stomach, such as the disintegration of food particles driven by physical and chemical processes, as well as variations in particle size distribution and particle morphology. In particular, detailed information about the disintegration of solid foods with different main components has been obtained from the GDS studies.

In food gastric digestion studies, the most reliable information is obtained from *in vivo* studies using human subjects, but it is difficult to conduct clinical experiments under various conditions, mainly due to restrictions on the number of subjects. There are growing needs for investigations using *in vitro* gastric digestion devices including the GDS, as much useful knowledge can be accumulated more readily by *in vitro* gastric digestion experiments with high reproducibility. An adequate combination of the *in vivo* and *in vitro* approaches (and *in silico* approach in some cases) is expected to lead to a better understanding of food gastric digestion. Further improvement and investigation of our GDS is needed to gain insights into the design of novel foods whose digestibility can be controlled based on life stage and health conditions. Promising applications of the GDS also include the design of care foods in a gradually increasing market for the elderly, for whom information about their gastric digestion remains insufficient. GDS technology could be utilized as a tool for developing well-designed care foods in the near future.

Acknowledgments

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